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Anti-inflammatory Constituents Isolated From *Launaea sarmentosa* Against Infection by LPS-stimulated Macrophages

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Abstract: Inflammation is one of the basic pathological processes due to the adaption of immune system against infection or irritation. Since non-steroidal anti-inflammatory drugs (NSAIDs) cause many side effects, investigation of anti-inflammatory natural products promising to provide novel therapeutic agents. Here, *Launaea sarmentosa* (Willd.) Kuntze, a medicinal herb applied to treat inflammation diseases, was investigated for its anti-inflammatory components to find new therapies for inflammatory syndromes. This study indicated that ethyl acetate fractional extract reduced the expression of pro-inflammatory cytokines, representing the highest activity for both NO radical-scavenging and NO secretion inhibition during LPS-stimulated RAW264.7 macrophages. Besides, five anti-inflammatory compounds, including succinic acid, quercetin, 2(4-hydroxyphenyl)acetic acid, luteolin-7-*O*-*β*-D-glucopyranoside, and quercetin-3-*O*-rutinoside, were isolated and elucidated the structure according to 1D and 2D- NMR. Among these compounds, succinic acid and 2(4-hydroxyphenyl)acetic acid were first reported in this species. Moreover, this study indicated that the presence of these compounds, typically quercetin and luteolin-7-*O*-*β*-D-glucopyranoside, enhanced anti-inflammatory ability via deactivation of NF-κB/MAPK pathway to mitigate the expression of IL-6. Hence, this study contributed the initial evidence of anti-inflammatory constituents from *Launaea sarmentose* and highlighted an approach to discovering natural items or phytotherapeutic agents.

Keywords: Anti-inflammatory; *Launaea sarmentosa*; mitogen-activated protein kinase; nuclear factor kappa B

1. Introduction

Inflammation is normally a self-protective physiological mechanism against a noxious stimulus to initiate tissue homeostasis from pathogens and injuries [1]. Dysregulation of inflammation has been

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recognized as a reason for pathophysiology leading to several chronic progressive diseases, including rheumatoid arthritis, atherosclerosis, type 2 diabetes, cardiovascular diseases, obesity, and especially cancer [2]. In the initiated immune activation, macrophages are the crucial immune cells that exert their role in the induction and regulation of inflammation. Indeed, macrophages activate inflammatory signaling cascades and release pro-inflammatory mediators and cytokines such as nitric oxide (NO), tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), etc. Lipopolysaccharide (LPS) is known as a potent trigger macrophage activator, which causes overexpression of pro-inflammatory mediators by activating Nuclear factor kappa B (NF-κB) and Mitogen-activated protein kinase (MAPK) signaling pathways [3]. As a result, extensive research on new agents that can inhibit the excessive production of inflammatory mediators on LPS-stimulated macrophages could be a promising therapeutic target for treating inflammation-related diseases.

Launaea sarmentosa (Willd.) Kuntze, a nutritious vegetable or creeping herb, was widely used in daily diets and as a Vietnamese folk remedy for treating diabetes or chronic inflammatory diseases [4]. The presence of active components belonging to flavonoids, alkaloids, carbohydrates, amino acids, and steroids indicated the role of *Launaea sarmentosa* in antioxidant, anti-inflammatory, and liver-protective activities [5]. Our previous study demonstrated the mechanism underlying its anti-inflammatory effects via activating NF-κB and MAPK signaling pathways [6]. Besides, chemical composition of *Launaea sarmentosa* was reported for supplementing the database of natural resources, as described in previous studies [7,8]. However, novel approaches and innovative strategies in drug development require new precursors or fully understood abilities, especially available natural products [9]. To date, to the limitation of our knowledge, no detailed report has explored the anti-inflammatory agents of *Launaea sarmentosa*. Thus, considering the necessity of the drug development, this study has continued to provide more evidence of anti-inflammatory constituents from *Launaea sarmentosa* through *in vitro* evaluation using RAW264.7 macrophage cells in LPS stimuli.

2. Materials and Methods

2.1. Plant Material

Launaea sarmentosa (LS) was harvested in Ben Tre province, Vietnam, in August 2022. The plants were botanically identified by Assoc. Prof. Dang Minh Quan deposited a voucher specimen (LS08.2022-BT002) in Laboratory of Medicinal Chemistry, CTU Hi-tech building, Can Tho University, Vietnam. The specimen was compared and recognized at National Plant Resources Center - Vietnam under a herbarium barcode: VNM00082022CTU.

2.2. Extraction Process and Isolation of Anti-inflammatory Compounds from Launaea sarmentosa

The aerial part of LS was collected, washed under tap water, and dried at 55 ºC. The sample was then ground using a waring two-Speed laboratory blender (Cole-Parmer, USA) to obtain dry powder and kept at -20 ºC for further extraction. The moisture content of dried powder was recorded as 5.08 ± 0.99%. The extraction process was utilized using an ultrasound-assisted method. Shortly, the dried powder was soaked in methanol (Merck, Germany) using an Elmasonic S100H ultrasonic water bath with a frequency of 40 kHz and 500 W nominal power. Next, all supernatants were centrifuged, filtered, and concentrated using Rotavapor R300 (BUCHI, Flawil, Switzerland) to obtain the crude methanol extract. Finally, LS methanol extract (LSm) was stored at 4 ºC for further experiments.

100 g LSm was suspended in an equal volume ratio of methanol and water. Then, the mixture was partitioned in ascending order of polarity organic solvents, producing fractions as *n*-hexane extract (Hex-ex, 24.6 g), ethyl acetate extract (EtOAc-ex, 8 g), and aqueous extract (Aq-ex, 12.4 g), respectively.

According to the results of NO experiment, ethyl acetate extract exerted its highest activity by suppressing nitric oxide production in LPS-induced inflammation. Thus, ethyl acetate extract was selected to be subject to silica gel-column chromatography (Silica gel 60; Merck, 230-400 mesh ASTM). A gradient of *n*-hexane, ethyl acetate, and methanol (Hex : EtOAc : Me, 100 : 0 : 0 to 0 : 0 :100, *v/v*)

was eluted to afford 10 sub-fractions. Each sub-fraction was repeatedly purified with a gradient of *n*-hexane and chloroform (Hex : $CHCl_3$, 100 : 0 to 0 : 100, *v/v*) or *n*-hexane-chloroform (Hex : $CHCl_3$, 5 : 95, *v/v*) or chloroform-methanol ($CHCl_3$: MeOH, 93 : 7, *v/v*) to obtain 5 compounds. These compounds were denoted as compounds **1**, **2**, **3**, **4** and **5** in corresponding yield as 22 mg, 121 mg, 15 mg, 185 mg and 32 mg, respectively. Thin-layer chromatography (TLC) plates pre-coated silica gel 60 with fluorescent indicator F_{254} (Merck, Germany) were conducted to check the purification. Visualization of TLC plates was carried out under UV light (254 and 365 nm) and then, using a mixture of ethanol with 5 % vanillin/H_2SO_4 (*v/v*) under heating in a hot plate to observe the spots.

2.4. Nitric oxide Radical-Scavenging Activity

The nitric oxide (NO) radical scavenging was accessed based on a previous report with slight modification [10]. An equal volume of 5 mM sodium nitroprusside and LSm extract or its fractional extract were mixed under exposing light for 30 min. Then, 100 µL of the mixture was added with 100 µL Griess-Romijn Nitrite reagent and incubated without light at room temperature for 10 min. The absorbance solution was recorded at 540 nm. The IC_{50} value (half-maximal inhibitory concentration) was used to evaluate the radical-scavenging efficiency.

2.5. Cell Viability

RAW264.7 cells (ATCC TIB-71^{TM}, Manassas, VA, USA) was maintained in a culture flask (25 cm^2) with Dulbecco's Modified Eagle's Medium (DMEM; GIBCO, Thermo Fisher Scientific, USA) contained 10% (*v/v*) fetal bovine serum (FBS; Hyclone, USA), 1 % penicillin-streptomycin and 2mM L-glutamine (Merck, Germany) under 5% CO_2 of a humidified atmosphere at 37 ºC.

Cell was seeded at a density of 2 x 10^5 cells/mL in 96-well plates for 24 h. Then, each sample was diluted in DMEM to obtain an appropriate concentration, adjusting 0.1 % (*v/v*) final concentration of Dimethyl sulfoxide (DMSO). Cell viability was analyzed using Cell Counting Kit-8 assay (CCK-8; Dojindo Molecular Technologies Inc., Rockville, USA). Subsequently, 10 µL of CCK-8 Kit was mixed with 90 µL DMEM and added to each cell group after removing media. Cells were pretreated with a serial concentration of sample for 24 h in the presence/absence of 1 µg/mL LPS (Merck, Germany). Finally, the optical density was measured at 450 nm using a microplate spectrophotometer (Epoch, BioTek, USA).

2.6. Determination of Nitric Oxide Production

Cells were pre-incubated with various concentrations of LSm, then washed in phosphate buffer saline (PBS), and stimulated in the presence of LPS (1 µg/mL) at 37 °C for 24h. The accumulation of nitric oxide (NO) was measured using Griess-Romijn Nitrite reagent (FUJIFILM-Wako, Japan) as described in our previous study [5]. Briefly, 100 µL of supernatant culture medium was mixed with 100 µL Griess reagent. The mixture was incubated without light exposure at room temperature for 10 min to develop color. The absorbance was measured at 540 nm using a microplate spectrophotometer compared with the standard curve of sodium nitrite ($y = 0.0034x + 0.0093$, $R^2 = 0.9992$). The IC_{50} value was used to evaluate the efficiency, while a positive control was 100 µM N-Nitro-**L**-arginine methyl ester (L-NAME, FUJIFILM-Wako, Japan).

2.7. Detection of Pro-Inflammatory Cytokines using Real-Time Quantitative Reverse Transcription PCR

Cells were incubated with the indicated concentration of LSm extract or purified compounds. After that, each sample was stimulated for 18 h in the presence/absence of LPS. Total RNA was purified using Qiagen RNeasy Kit (Qiagen, Germany) following the manufacturer's instructions. Then, the

mRNA expression was analyzed using RT-*q*PCR as mentioned in our previous report [10]. *β*-actin, as a normalizing control. All primers were presented in Supporting Information.

2.8. Western Blotting

Cells were pretreated with appropriate concentrations of selected compound prior to LPS stimulation for 24 h. Subsequently, whole proteins were extracted using a RIPA buffer (FUJIFILM-Wako, Japan). After that, BCA protein assay (Thermo Fisher Scientific, USA) was used to determine the protein concentration before diluting the equivalent concentration for each sample. Next, samples were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE, 10%, *v/v*) and then transferred onto PVDF membranes. The membrane was blocked using OneStep Blocker (Bio-Helix, Taiwan) for 1 h. Finally, samples were incubated with the primary antibody (anti-phospho-p38; anti-phospho-p65; Sigma-Aldrich, USA). The visualized bands were observed using SuperSignal West Atto ultimate sensitivity substrate (Thermo Fisher Scientific, USA).

2.9. Structure Elucidation Experimental Procedure

The structure elucidation of samples was analyzed on Bruker Avance III-600 MHz (Bruker, Rheinstetten, Germany) with CD_3OD, DMSO-d_6.

2.10. Statistical Analysis

All value are presented as the mean ± standard deviation (S.D.), at least three independent replications. One-way ANOVA was used to indicate the statistical significance of groups ($p < 0.05$) and performed using v8.0.1 GraphPad Prism software (GraphPad Software Inc., CA, USA).

3. Results and Discussion

3.1. Effect of LSm and Its Fractions on Inflammatory Biomarkers

CCK-8 test was performed to determine each extract's cytotoxicity and evaluate the effective concentration with minimum toxicity. LPS triggers macrophage, leading to cell damage via apoptosis and pyrosis, thereby suppressing cell viability and changing the morphological cell [11,12]. The results indicated that the cell survival of LSm and its fraction was not substantially different from control group up to 200 µg/mL, suggesting that these concentrations were not toxic (Table S2).

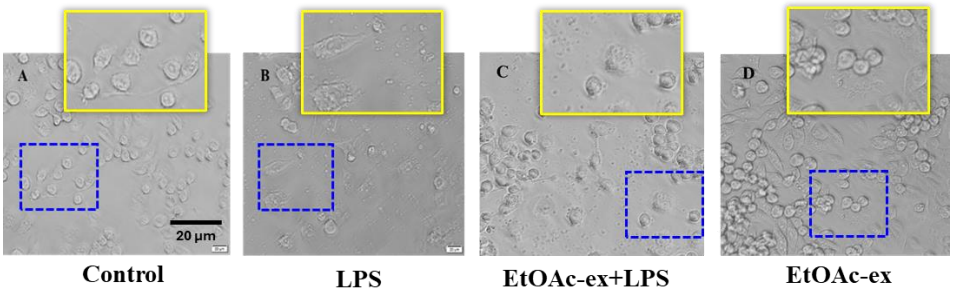


Figure 1. Cell morphology was observed with ethyl acetate fractional extract in LPS-stimulated. (A). Control group without LPS and extract; (B). Cell was treated with LPS for 8 h; (C). The cell was pretreated with 200 µg/mL ethyl acetate fraction (EtOAc-ex), followed by the presence of LPS for 8 h; (D). Cell was treated alone with 200 µg/mL ethyl acetate fraction extract (EtOAc-ex)

In contrast, the cell viability after being exposed to 400 and 800 µg/mL was reduced by over 10 % than the control group. Moreover, the cell viability was improved considerably by co-incubation with these concentrations in LPS-stimulated. Besides, the morphology transformation of the cell as spindle-shaped pseudopodia was observed upon LPS activation, whereas this shape was attenuated by pre-treatment of extracts, as shown in Figure 1 [13]. Thus, these results demonstrated that LSm and its fraction extracts defend against LPS-induced cell damage.

Nitric oxide (NO), as a ubiquitous signaling molecule, is one of the key mediators in physiological and pathophysiological processes related to apoptosis or stress response in many cell types, especially inflammatory cells [14]. Quantitative of exhaled nitric oxide or fraction of exhaled nitric oxide is a clinical test to assess airway inflammation in asthma [15]. Moreover, abnormally high NO production in hepatic failure and sepsis could improved through removing excess NO [16]. Indeed, two-way approaches for suppressing NO levels include reducing NO synthesis and using NO scavengers [17]. Therefore, the effect of LSm and its fractions was investigated via NO radical-scavenging activity and reducing NO production in LPS-stimulated macrophages.

Table 1. Nitric oxide radical-scavenging activity of LSm and its fractional extract

Sample	Nitric oxide inhibitory activity			
	LSm	**Hex-ex**	**EtOAc-ex**	**Aq-ex**
IC_{50} (µg/mL)	206.6 ± 2.80^{a}	319.6 ± 8.5^{b}	224.4 ± 0.8^{c}	887.38 ± 28.6^{d}

Different superscript letters in each column depict a significant difference value ($p < 0.05$)

The NO inhibitory efficiency of each extract was evaluated according to IC_{50} value, expressed as the minimum concentration required to scavenge initial free radicals by 50%. As depicted in Table 1, the ethyl acetate fraction (EtOAc-ex) exerted a higher nitric oxide radicals-scavenging activity than other fractions, reaching IC_{50} value of 224.4 µg/mL. This phenomenon could be explained by the high presence of active components, which were concentrated in the extract due to fractionation [18]. However, the highest activity was observed in crude extract (LSm) with IC_{50} of 206.6 µg/mL, suggesting that LSm was more active than its fractions. Typically, the effect of active ingredients is masked by other inactive compounds in a complex called antagonism, which also appears in natural product mixtures [19]. Notably, the hexane fraction (Hex-ex) also exhibited interesting NO scavenging activity, which revealed an IC_{50} value of 319.6 µg/mL, while the aqueous fraction had weak scavenging activity (IC_{50} value of 887.38 µg/mL). Therefore, the effect of active ingredients could be attributed to the presence of non-polar and moderately polar compounds through the fractionation process. These results indicated that the observed NO scavenger of LSm and its fractions can be attributed to flavonoids and terpenoid groups, consistent with a previous study [20].

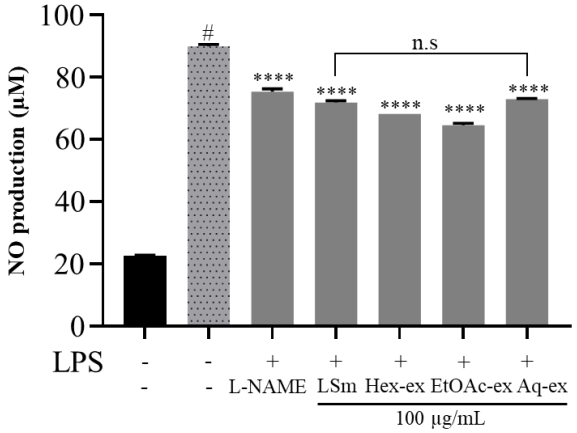


Figure 2. Effect of LSm and its fractions on NO production under LPS-stimulated RAW264.7
Data are described as the mean ± S.D (n=6); n.s, not significant; #, $p<0.05$ vs. control group without LPS; ****, $p<0.0001$ vs. LPS group

Regarding the effect of LSm and its fraction on NO production, Figure 4 shows that NO generation was significantly enhanced during LPS stimulation. Indeed, immune cells release NO and pro-inflammatory mediators in response to inflammation caused by LPS after infection. Therefore, excessive NO secretion is one of the manifestations leading to inflammation [21]. As illustrated in Figure 4, pre-treatment with LSm and its fraction decreased the NO production compared to the LPS group, whereas the NO level was remarkably suppressed in the EtOAc-ex group than LSm and other fractions, indicating its potential in anti-inflammatory response. Hence, for both the NO radical-scavenging activity and the inhibition of NO secretion during LPS-induced inflammation, EtOAc-ex exerts its role as a potential candidate for further investigation of existing active components.

To confirm the inhibition efficiency of EtOAc-ex on pro-inflammatory cytokines, the levels of TNF-α and iNOS were analyzed using *q*RT-PCR. The sustained NO production is first highlighted as the inducible form of nitric oxide synthase (iNOS) expression [22]. At the same time, TNF-α, a central cytokine, promotes inflammatory reactions directly by inducing the expression of the inflammatory genes and indirectly causing cell death [23]. By up-regulating the mRNA expression of these genes was demonstrated since LPS-activated macrophages, while it was down-regulated upon the presence of EtOAc-ex (Figure 3). This result is consistent with our previous report [6]. Collectively, EtOAc-ex can reduce pro-inflammatory cytokines, suppressing NO production, which proves that it may effectively alleviate the progression of inflammatory diseases. For further evaluation of new therapeutics, EtOAc-ex was chosen to isolate active compounds and investigate their underlying inflammation response mechanisms.

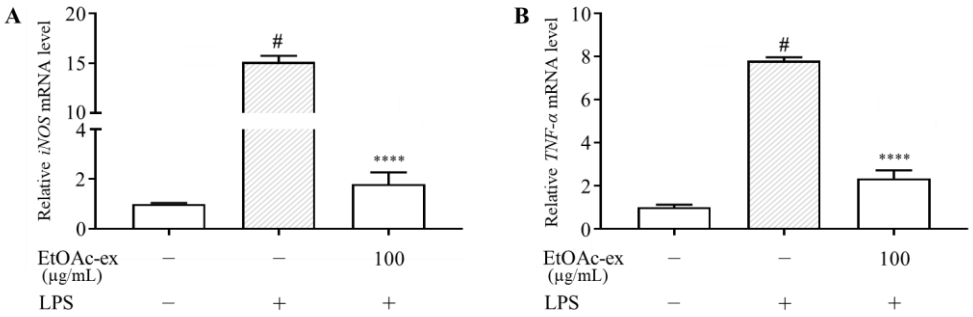


Figure 3. Effect of EtOAc-ex on pro-inflammatory cytokines in LPS-activated macrophages. (A) mRNA *iNOS* expression in LPS-induced inflammation was pretreated with 100 μg/mL of EtOAc-ex. (B) mRNA *TNF-α* expression in LPS-induced inflammation was pretreated with 100 μg/mL of EtOAc-ex. Data were presented as the mean ± S.D (n=4); #, $p<0.05$ vs. control group without LPS; ****, $p<0.0001$ vs. LPS group

3.2 Determination of Active Compounds from EtOAc-ex Against LPS-induced Inflammation on Macrophages

Regarding the existence of anti-inflammatory compounds from EtOAc-ex, fine compounds were isolated using column chromatography. Then, for the confirmation of anti-inflammatory effectiveness, these samples were evaluated for their role in inflammation markers and inflammatory underlying mechanisms. Firstly, the cytotoxicity of these compounds was accessed at concentrations of 1 and 10 μg/mL. As expected, cell survival was not significantly different from that of the control group, indicating that it was non-toxic at those concentrations (data not shown). Moreover, pre-treatment with these compounds consequently decreased the NO production within LPS-induced inflammation, except 1 μg/mL of compounds **1** and **2** (Figure 4A). Notably, the level of NO secretion was dramatically reduced in the presence of 10 μg/mL compounds **2** and **4**, reaching approximately 50 and 60%, respectively. Secondly, Interleukin-6 (IL-6) mRNA expression level, a trans-signaling pathway associated with immunomodulatory, was investigated in the presence of compounds 2 and 4 [24]. The IL-6 expression in LPS-induced inflammation occurred later than TNF-α and Interleukin-1β (IL-1β) [25]. The role of IL-6 was reported as a prototype cytokine with diverse functions on acute inflammation

and adaptive immune response. Conversely, overexpression of IL-6 is also related to the acute development and severe inflammation associated with systemic inflammatory response syndrome (SIRS) and cytokine release syndrome (CRS) [25]. As depicted in Figure 4B, compounds **2** and **4** significantly downregulated the mRNA IL-6 level by 5.5 and 4.6-fold, respectively. Finally, these results indicated that the presence of these compounds mitigated the expression of targets NO and IL-6, suggesting their protective role against LPS-induced inflammation.

To get an in-depth grasp of their underlying inflammation mechanisms, the expression levels of phosphorylated-NF-κB p65 (p-p65) and p-p38 MAPK proteins, vital phosphorylation of downstream subunits associated with inflammation pathways, were evaluated using Western blot. Under response to inflammatory stimuli such as LPS exposure, NF-κB signaling is activated, leading to IκB degradation and phosphorylation of p65. In there, NF-κB heterodimers, including p50 and p65, are quickly liberated and nuclear translocation occurs to promote the expression of inflammatory cytokines such as TNF-α, IL-6 etc [26,27]. Furthermore, NF-κB signaling also exerts its role in physiological and pathological processes due to direct or indirect regulation with another factor, thereby triggering the interaction with other signaling pathways. Noteworthy, the transcriptional specificity of NF-κB is related to the interaction with MAPK. Among the regulation proteins belonging to MAPK signaling pathway, p38 MAPK mediated the decisive role in inflammation, and its targeted deletion could impair po-inflammatory cytokines expression. Indeed, Mitogen- and stress-activated kinases (MSK) 1 and 2 are activated downstream through the phosphorylation of p38 MAPK, leading to regulate the transcription of inflammatory genes [28–30]. As illustrated in Figure 4C, adding compounds **2** and **4** to LPS-stimulated macrophage dramatically suppressed the phosphorylation of p65 and p38 MAPK, respectively, while LPS-treated alone enhanced these protein levels. This demonstrated that the existence of these active compounds typically compounds **2** and **4**, deactivated the MAPK and NF-κB signaling pathway in LPS-induced inflammation.

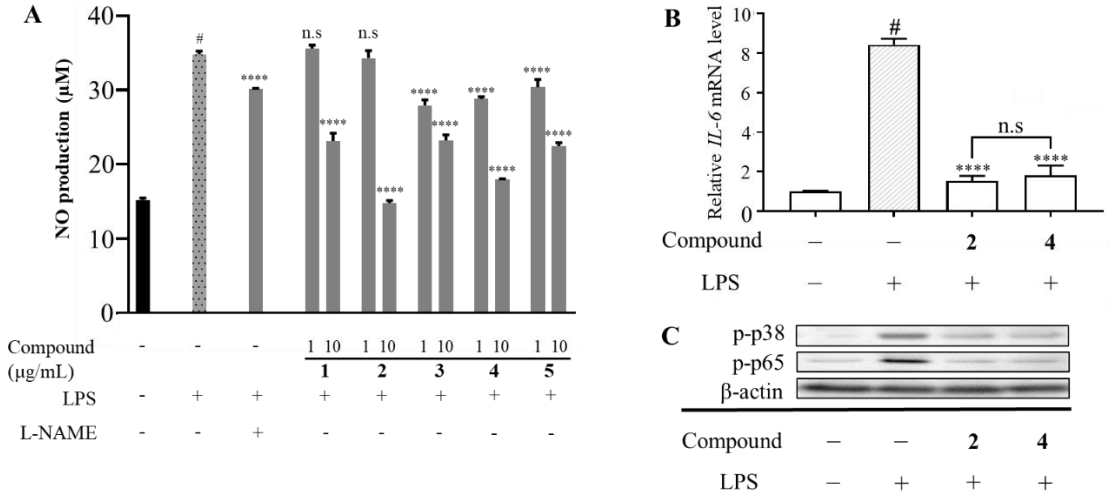
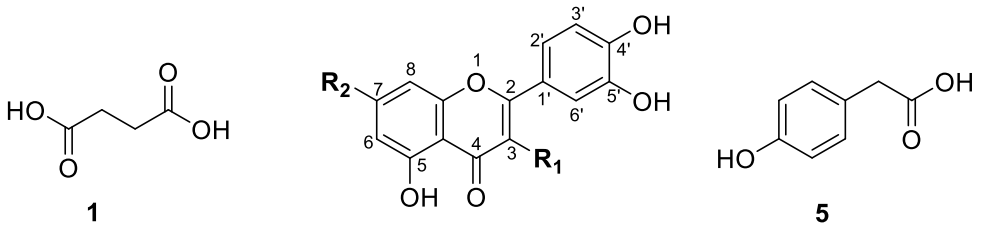


Figure 4. Effect of active compounds on inflammatory biomarkers in LPS-activated macrophages. (A) NO inhibition was obtained with 1 and 10 µg/mL of each compound, followed by treatment with LPS. (B) mRNA *IL-6* expression level with 1 and 10 µg/mL of compounds **2** and **4** was evaluated by *q*RT-PCR. (C) Expression of p-p65 and p-p38 following the treatment of 10 µg/mL of compounds **2** and **4** was determined using Western blot (n=3). Data were presented as the mean ± S.D (n=4); n.s, not significant; #, $p<0.05$ vs. control group without LPS; ****, $p<0.0001$ vs. LPS group

Otherwise, the structure of all isolated compounds was analyzed using 1D and 2D-NMR and compared with the literature published (Supporting Information). As shown in Figure 5, the structure of isolated compounds was initially elucidated and numbered, including succinic acid (**1**), quercetin (**2**),

quercetin-3-*O*-rutinoside (**3**), luteolin-*7-O-β*-D-glucopyranoside (**4**) and 2(4-hydroxyphenyl)acetic acid (**5**), respectively. Notably, compounds (**1**) and (**5**) were first isolated from this species.



2 R_1= OH, R_2=OH
3 R_1= O-Rut, R_2=OH
4 R_1= H, R_2=O-Glc
Rut = rutinose; Glc = glucose

Figure 5. Structures of active compounds isolated from EtOAc-ex of *Launaea sarmentosa*

Correspondingly, to clarify the effect of these isolated compounds, the IC_{50} value according to the percentage of NO release from LPS-stimulated macrophage was investigated. Here, quercetin (compound **2**) and luteolin-7-*O-β*-D-glucopyranoside (compound **4**) exhibited the most substantial reduction of NO release at IC_{50} values of 27.44 µM and 30.38 µM, respectively, consistent with initial evaluation (Table S3). These results were similar to a previous report, which indicated the IC_{50} value of quercetin around 25 µM. However, our data presented a higher IC_{50} value of luteolin-7-*O-β*-D-glucopyranoside than this report, whereas the inhibition percentage was about 38.6 % at 100 µM [32]. In particular, quercetin (compound **2**), belonging to the flavonols group, indicated stronger activity than its derivatives as quercetin-3-*O*-rutinoside (compound **3**) due to the presence of hydroxy group (-OH) at position 3 in ring C. Moreover, the glycoside linkage appearance in the structure of luteolin-7-*O-β*-D-glucopyranoside (compound **4**) also slightly reduced activity compared to quercetin. It could be explained that the hydroxyl and a bulky group substitutions on ring A- and B- might lowered the NO inhibitory activity [31,32]. Conversely, succinic acid (compound 1), 2(4-hydroxyphenyl)acetic acid (compound **5**), and quercetin-3-*O*-rutinoside (compound **3**) displayed limitation inhibitory with IC_{50} over 100 µM. However, succinic acid and its derivatives were reported as a primary hypoglycemic and potential immunomodulatory agent [33]. Controlled and sustained succinic acid can enhance cellular energy supply and improve the prognosis of sepsis via epigenetic changes, which is a promising therapeutic for sepsis [34]. Besides, 2(4-hydroxyphenyl)acetic acid, a phenolic acid isolated from *Aster tataricus*, significantly attenuated inflammation and edema in seawater aspiration-induced lung injury from rats via inhibiting hypertonic and hypoxic induction of HIF-1α [35]. It could be revealed as a supplement dietary agent for hepatoprotective [36]. In accordance with above results, quercetin and luteolin-7-*O-β*-D-glucopyranoside exerted their roles in the inflammatory responses by modulating the activation of NF-κB and MAPK. Indeed, quercetin strongly inhibited the NF-κB phosphorylation, then blocked p65 NF-κB translocation, thereby suppressing inflammatory reaction via p38 MAPK signal transduction, as mentioned in previous studies [37,38]. Similarly, luteolin and its aglycone or glucoside, especially luteolin-7-*O-β*-D-glucopyranoside, also potentially attenuated inflammation through the deactivation of NF-κB/AP-1/PI3K-Akt pathway in LPS-activated macrophages [39,40]. In addition, luteolin-7-*O-β*-D-glucopyranoside confirmed the anti-apoptosis effect due to declining phosphorylated-p38 MAPK, representing a candidate for preventing myocardial infarction symptoms [41]. Remarkably, the presence of the glucose group confers differences in chemical structure, leading to diversity in bioactivity. Conversely, this property improves the hydrophilicity of the compound and is a potential agent for drug development based on the possibility of glucose transporters [42].

Currently, the main components authenticated from the essential oil of *Launaea sarmentosa* exhibited lower free energy of binding with COX-1 and COX-2 than diclofenac via *in silico* basis. This hypothesis contributed more evidence for its anti-inflammatory properties [43]. Additionally, the changes in natural ingredients during two distinct seasonal climates from *Launaea sarmentosa* are associated with the inhibition of *Candida*, promising to develop new antifungal agents [44]. Our findings imply that the presence of these constituents, belonging to plant secondary metabolites, contributed to

the diversity of bioactive activity related to inflammation response. In conclusion, our results provide the initial evidence of anti-inflammatory constituents from *Launaea sarmentosa* and highlight an approach for discovering natural items or phytotherapeutic agents.

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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